

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/GB2004/004707

International filing date (day/month/year)
08.11.2004

Priority date (day/month/year)
07.11.2003

International Patent Classification (IPC) or both national classification and IPC
C07H21/00, C12Q1/68, C12N15/11

Applicant
SOLEXA LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for International preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

80/578460

International application No.
PCT/GB2004/004707

Box No. I Basis of the opinion

AP20 Rec'd PCT/PTO 08 MAY 2006

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ in written format
 - ☐ in computer readable form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/GB2004/004707

Box No. II Priority

1. ☐ The following document has not been furnished:
- ☐ copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
 - ☐ translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).
- Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	14,16,17,35,36,40
	No: Claims	1-13,15,18-34,37-39
Inventive step (IS)	Yes: Claims	
	No: Claims	1-40
Industrial applicability (IA)	Yes: Claims	1-40
	No: Claims	

2. Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

PCT/GB2004/004707

The present application describes a hairpin oligonucleotide characterised in that a sulfur-based nucleophile is attached to an internal nucleotide in the hairpin through a linker to enable binding to a solid support.

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

- D1: WO 97/08183 A1 (LANE, MICHAEL, J; BENIGHT, ALBERT, S; FALDASZ, BRIAN, D) 6 March 1997
- D2: ZHAO XIAODONG ET AL: "Immobilization of oligodeoxyribonucleotides with multiple anchors to microchips" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 29, no. 4, 15 February 2001, pages 955-959
- D3: EP-A1-0 174 879 (INSTITUT PASTEUR; CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE ; CENTR) 19 March 1986
- D4: US 2003/022207 A1 (BALASUBRAMANIAN SHANKAR ET AL) 30 January 2003
- D5: WO 01/92284 A1 (AMERSHAM PHARMACIA BIOTECH UK LIMITED; ODEDRA, RAJ; SIMMONDS, ADRIAN;) 6 December 2001

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-13,15,18-34,37-39 is not new in the sense of Article 33(2) PCT.
- 1.1 The document D1 discloses (the references in parentheses applying to this document): hairpin polynucleotides having biotin attached to an internal nucleotide in the hairpin through a linker to enable binding to a solid support (pages 9 - 10).

Thus, the subject-matter of claims 1,2 is not new in the sense of Article 33(2) PCT.

- 1.2 The document D2 discloses (the references in parentheses applying to this document): hairpin polynucleotides (the whole document) comprising phosphorothioates in the loop and being attached to glass coated with bromoacetamidopropylsilane (Fig. 1), hybridization (page 957, right-hand column - page 958, left-hand column).

Thus, the subject-matter of claims 1-10,15,18-20,23-34,37-39 is not new in the sense of Article 33(2) PCT.

- 1.3 The document D3 discloses (the references in parentheses applying to this document): hairpin polynucleotides having a sulfur-based nucleophile attached to an internal nucleotide in the hairpin through a linker to enable binding to a solid support (pages 4-11), DNA or RNA (page 9, paragraph 1), comprising diethylen glycol (page 5, paragraph 3), chain comprising amine (page 7), amide (page 14), glass beads (page 6, paragraph 1), electrophilic group attached to silicon atom (page 17).

Thus, the subject-matter of claims 1,2,8,11-13,19,20,23-31,33,34,37-39 is not new in the sense of Article 33(2) PCT.

- 1.4 The document D4 discloses (the references in parentheses applying to this document): the immobilization of polynucleotides onto the surface of a support (the whole document) for example via hairpin polynucleotides formed from 2 separate polynucleotides with complementary regions and a connecting non-nucleotidic loop comprising PEG (page 5, paragraph 53).

Thus, the subject-matter of claims 21,22 is not new in the sense of Article 33(2) PCT.

- 2 Claims 14,16,17,35,36,40 formally meet the requirements of Article 33(2) PCT because their subject-matter was not disclosed in the available prior art.
- 3 Even if the novelty objections stated above could have been overcome, the present application still would not have met the criteria of Article 33(1) PCT, because the subject-matter of claims 1-40 does not involve an inventive step in the sense of Article 33(3) PCT.

The document D3 is regarded as being the closest prior art to the subject-matter of claims 1-40, and makes the disclosures as stated above.

The subject-matter of the present application differs from document D3 in that: different sulfur-based nucleophiles were used.

The problem to be solved by the present invention may therefore be regarded as providing further sulfur-based nucleophiles for the attachment of hairpin polynucleotides to a solid support.

The solution to this problem proposed by the present application consists of the provision of sulfur-based nucleophiles as depicted in formula (I) in claims 3-7 of the present application for the attachment of hairpin polynucleotides to a solid support.

Regarding the subject-matter of claims 1-13,15,18-20,23-34,37-39:

D3 discloses the use of sulfur-based nucleophiles for the attachment of oligonucleotides, including hairpin oligonucleotides, to a solid support (e.g. see pages 9 and 10).

D2 also discloses the attachment of oligonucleotides to a solid support -in this case via phosphoorthothioate (the whole document)- and mentions that a linker can improve hybridization (page 955, left-hand column, paragraph 1).

Thus, in the light of the teaching of D3 the use of a linker with another nucleophile such as an phosphoorthothioate disclosed in D2 would be an obvious choice for the person skilled in the art, and does not involve an inventive step (Article 33(3) PCT).

Regarding the subject-matter of claims 21,22:

D4 makes the disclosure as stated above. D4 also discloses that the loop of the hairpin can be formed by nucleotides or by a linker moiety such as PEG (page 5, paragraph 53).

Regarding the subject-matter of claim 14:

The use of a different linker, such as for example a propargyl-based linker is also an obvious choice for a person skilled in the art, for example document D5 discloses propargyl-based compounds for the linkage of nucleotide analogues to a reporter (the whole document, e.g. claim 12). Furthermore, D5 discloses the use of the latter for immobilised hairpin oligonucleotides (page 12, lines 23-25).

**WRITTEN OPINION OF THE
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International application No.

PCT/GB2004/004707

Regarding the subject-matter of claims 16,17,35,36,40:

The use of hairpin oligonucleotides for screening for example human genomic DNA and for sequencing is also obvious to the person skilled in the art.

Thus, the subject-matter of claims 1-40 does not involve an inventive step (Article 33(3) PCT).

- 4 The subject-matter of claims 1-40 is susceptible of industrial application (Article 33(4) PCT).